

Homatula gelao (Cypriniformes, Nemacheilidae), a new nemacheiline species of loach from the upper Yangtze River basin in Guizhou Province, southwestern China

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Abstract

Homatula gelao, a new species, is here described from the Wu-Jiang of the Yangtze River basin in Guizhou province, southwestern China. This new species belongs to the elongate-bodied group of *Homatula* defined by having a complete lateral line, sparsely scales hidden in skin on the predorsal body, and a slender body with a uniform depth. It, along with *H. variegata*, is distinguished from all other species of this group in having a broadly rounded caudal fin and a small number of scales sparsely scattered over the predorsal body. Both differ in body coloration, striped pattern on the predorsal body, and caudal-peduncle length. The validity of the new species is corroborated by distinct genetic distance divergence with closely related congeneric species and its monophyly recovered in a mtDNA COI gene-based phylogenetic analysis. It is separated from other *Homatula* species by a minimum of 8.2% Kimura 2-parameter distance in the COI gene.

Key Words

Homatula gelao, new species, morphological comparison, taxonomy, Yangtze River basin

Introduction

The Furong-Jiang, the largest tributary on the western bank of the middle Wu-Jiang in Guizhou province, Southwest China, rising in the eastern foothills of the Dalou Mountains. It has a total main channel length of 230 km, with a total fall of 1100 m in its course and an average slope of about 4.8‰, covering a drainage area of roughly 7,360 km². The Furong-Jiang runs through

the Wuling Mountains, and its upper reaches divided into two tributaries, the Qingxi-Jiang and the San-Jiang. The Qingxi-Jiang rises in Huanglian Township in eastern Tongzi County. It flows southeast through Suiyang County and joins the Furong-Jiang at Chuangpujing Village in Zheng'an County. The river has a drainage area of 1,494 km², a main channel length of 78 km, and an average slope of about 9.23‰. The San-Jiang rises in the eastern foothills of Dalou Mountain in Boshulin

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Town, Nanchuan County. It flows southeast to join the Furong-Jiang on the right bank of Qunle Village. The river covers a drainage area of 1,036 km², with a main channel length of around 75 km and an average slope of around 6.8‰. The Furong-Jiang then proceeds to enter Pengshui County, Chongqing Municipality, where it makes an “S”-shaped bend before joining the Wu-jiang on the left bank of Jiangkou Town, Wulong County.

Up till now, few specific surveys have been conducted into the fish diversity of the Furong-Jiang. Despite many species so far reported from the Furong-Jiang, their names were borrowed from those of the upper Yangtze River in general or the rest of the Wu-Jiang in particular (Zheng and Li 1986; Xie et al. 2005; Yang et al. 2017). Recently, the integration of morphological and molecular data demonstrated that specimens, currently identified as *Acrossocheilus yunnanensis* (Regan 1904), collected from this river represented an undescribed species, thus named as *A. furongjiangensis* (Xiao et al. 2024). The description of this new species implies that the fish diversity of the Furong-Jiang is still poorly understood. This is further evidenced by the recent discovery of a new *Homatula* species from the same river.

Homatula is so far represented in Guizhou, in the upper Yangtze River basin, belonging to the *H. variegata*-like species of the genus (Liu 2022), with the exception of the *H. potanini* in the Chishui River (Wang et al. 2017). Only the *H. variegata* has been reported from the tributaries of the Furong-jiang in the Wu-jiang basin (Yang 2022). However, *H. variegata* has recently been shown that the species is truly represented by the population of the Wei-He flowing into the Yellow River (or Huang-He in Mandarin Chinese) basin (Liu et al. 2022). The integration of morphological and molecular data indicated that the Furong-Jiang specimens, currently reported as *H. variegata*, conform to none of all valid species of this genus and thus represent an undescribed species, herein named as *H. gelao* sp. nov.

Methods

Study area and sampling

All specimens were collected from the Huaixi-He, a stream tributary to Furong-Jiang flowing into the Wu-Jiang (28°08'N-28°20'N, 107°02'E-107°52'E), in Huaiping Village, Jiucheng Town, Daozhen Gelao (The Chinese common name is “仡佬”) and Miao (The Chinese common name is “苗族”) Autonomous County, Guizhou Province, China (Fig. 1). Each caught individual was contained in a plastic container, transported to a field laboratory, and examined within 30 minutes after euthanized with buffered tricaine methanesulfonate (MS-222). After examination, the muscles were removed from some collected specimens, transferred in liquid nitrogen, and stored at -80 °C for further molecular analyses.

Their voucher specimens, along with the rest of the captured specimens, were firstly fixed in 10% formalin and then stored in 75% alcohol liquid for permanent preservation. All collected specimens are housed in the collection room at the Zunyi Vocational and Technical College (ZVTC), Zunyi, Guizhou Province, China.

Morphological measurement and analysis

All measurements and counts were taken following Kottelat's (1990) methods. Morphometric measurements were made with digital calipers to the nearest 0.01 mm from the left side of the body. The standard length is from the snout tip to the base of the caudal fin. Body depth was measured at the origin of the dorsal fin, while eye diameter was measured horizontally. Lateral head length was measured from the tip of the snout to the posterior margin of the operculum, excluding the operculum membrane. Micro-computed tomography (Micro-CT) images were used to observe skeletal structures and count vertebrae and fin rays. Weberian apparatus was counted as four vertebrae.

In light of the fact that *H. gelao* was previously misidentified as *H. variegata*, consequently, principal component analysis (PCA) was employed to analyze the morphological variations between *H. gelao* and *H. variegata*. The data of *H. variegata* used for principal component analysis (PCA) comparison with new specimens was quoted from Liu et al. (2022). Prior to performing the PCA, the KMO and Bartlett tests for sphericity were performed on 17 morphological traits of *H. gelao* and *H. variegata*, and the PCA was processed with log-transformed data in percent of standard length (SL) in a covariance matrix for analysis. The number of extracted principal components (PCs) was reduced to three.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was isolated from muscles on the left side of the predorsal body above the lateral line using the Genome DNA Extraction Kit (Sangon Biotech, China). The cytochrome oxidase I (COI) gene was used for molecular phylogenetic analyses. A fragment of this gene was amplified by polymerase chain reaction (PCR) with the following primers: the forward primer, 5'-TCAACCAAC-CACAAAGACATTGGCAC-3', and the reverse primer, 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward et al. 2005). After an initial 2 minutes of denaturation at 94 °C, the reaction mixture was subjected to 35 cycles of denaturing at 94 °C for 30 s, annealing at 55 °C for 30 s, and extending at 72 °C for 30 s, followed by a 2-minute extension at 72 °C. The amplification products were purified and sequenced using the ABI3730 XL DNA System (Apep Biosystems Trading (Shanghai) Co., Ltd.).

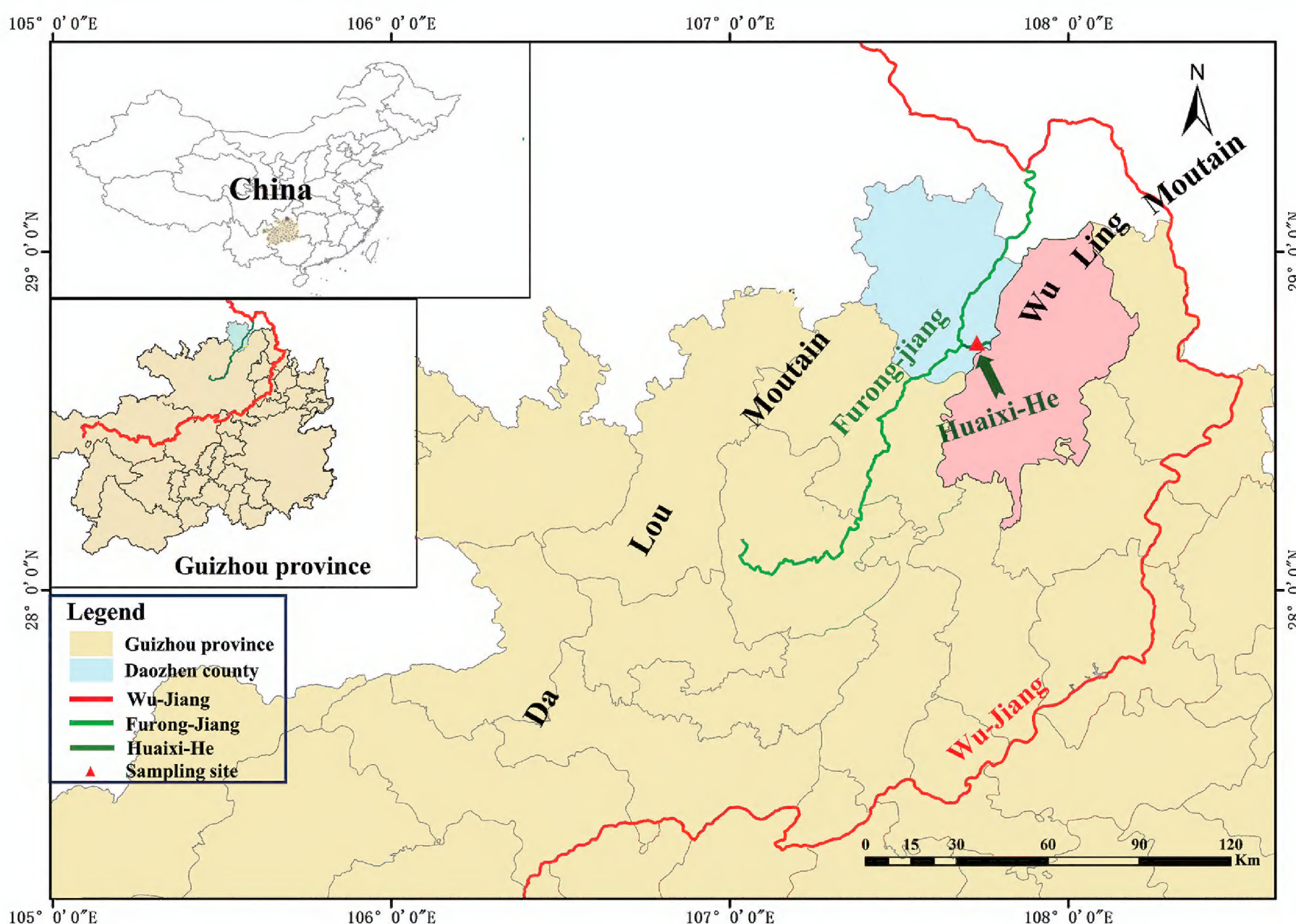


Figure 1. Map showing the sampling site of the type specimens of *H. gelao* (red triangle symbol).

Phylogenetic analyses

COI sequences of nine specimens of new species were amplified for this study. Sixty-five COI gene sequences from 16 species of *Homatula* were downloaded from GenBank (Table 1). Also retrieved from GenBank were two sequences of the same gene from *Schistura latifasciata* (MF953223) and *S. porthos* (MF953224), which were chosen as the outgroups for phylogenetic analysis (Table 1). These sequences were aligned with those of the specimens determined for the new species in this study. The divergences between lineages were calculated according to the Kimura two-parameter model (K2P) (Kimura 1980) using MEGA 7.0 (Kumar et al. 2016). The neighbor-joining tree (Saitou and Nei 1987) was generated using MEGA 7.0 with the Kimura two-parameter model. Bootstrap analyses were based on 1000 replicates. Both missing data and gaps were treated as missing data.

Results

Homatula gelao Xiao, sp. nov.

<https://zoobank.org/0A53E75E-4DEA-442D-A0AA-5B8E66662CAD>

Holotype. • ZVTC 20200601, 127.56 mm SL. Guizhou Province: Zunyi City: Daozheng County: Huaixi-He, a stream tributary to Furong-jiang of Wujiag basin, at Huaiping

Village (28°43'39.2124"N, 107°45'2.7072"E, roughly 632.5 m above sea level) of Jiucheng Town; collected by G. B. Xiao, Gui Bang Xiao, 06 January 2020 (Fig. 2).

Paratypes. • ZVTC 20200602–ZVTC 202006029, 28 specimens, 98.3–135.5 mm SL, other data same as holotype.

Diagnosis. A member of the elongate-bodied group of *Homatula* defined by having a slender body with a uniform depth (9.2%–14.4% of SL), a complete lateral line, and a scarcely scaled or scaleless predorsal body. *H. gelao* resembles *H. variegata* in the presence of a broadly rounded (vs. truncate or obliquely truncate) caudal fin and scales sparsely scattered on the predorsal body (vs. predorsal body unscaled or sparsely scaled on the dorsum and flank), which distinguishes both from all other species of this group (*H. berezowskii*, *H. guanheensis*, *H. laxiclathra*, *H. longidorsalis*, *H. oxygnathra*, and *H. tigris*). It differs from *H. variegata* in possessing a body coloration of 17–18 brown vertical bars narrower than interspaces, particularly on the Caudal peduncle flank (vs. dense, undulating vertical bars slightly wider or equal to interspaces); a thick brown-black stripe running along dorsal midline from behind head to dorsal fin origin; and symmetrical black vertical bars on sides of body (vs. light brown spots on the dorsum and irregular markings on the sides of the body); and a longer (vs. equal) distance from the origin of the anal fin to the origin of the pelvic fin than the distance from the origin of the anal fin to the base of the caudal fin.

Table 1. The COI sequences utilized for phylogenetic analysis in this study.

Taxon	Voucher number	GenBank number	Resource
<i>Schistura latifasciata</i>	KIZ:20150096	MF953223	(Endruweit et al. 2018)
<i>Schistura porthos</i>	KIZ:2010003121	MF953224	(Endruweit et al. 2018)
<i>Homatula anguillioides</i>	KIZ:2008005997	MF953178	(Endruweit et al. 2018)
<i>Homatula anguillioides</i>	KIZ:2008005998	MF953179	(Endruweit et al. 2018)
<i>Homatula anguillioides</i>	KIZ:20080302	MF953180	(Endruweit et al. 2018)
<i>Homatula anguillioides</i>	KIZ:20080303	MF953181	(Endruweit et al. 2018)
<i>Homatula anguillioides</i>	KIZ:20080304	MF953182	(Endruweit et al. 2018)
<i>Homatula anguillioides</i>	KIZ:20080305	MF953183	(Endruweit et al. 2018)
<i>Homatula anguillioides</i>	KIZ:20080306	MF953184	(Endruweit et al. 2018)
<i>Homatula pycnolepis</i>	KIZ:20090067	MF953202	(Endruweit et al. 2018)
<i>Homatula pycnolepis</i>	KIZ:20090073	MF953203	(Endruweit et al. 2018)
<i>Homatula pycnolepis</i>	KIZ:20100200	MF953204	(Endruweit et al. 2018)
<i>Homatula pycnolepis</i>	KIZ:20100202	MF953205	(Endruweit et al. 2018)
<i>Homatula pycnolepis</i>	KIZ:20100203	MF953206	(Endruweit et al. 2018)
<i>Homatula pycnolepis</i>	KIZ:20100204	MF953207	(Endruweit et al. 2018)
<i>Homatula potanini</i>	KIZ:2010000236	MF953198	(Endruweit et al. 2018)
<i>Homatula potanini</i>	KIZ:2010000237	MF953199	(Endruweit et al. 2018)
<i>Homatula potanini</i>	KIZ:2010000279	MF953200	(Endruweit et al. 2018)
<i>Homatula potanini</i>	KIZ:2010000280	MF953201	(Endruweit et al. 2018)
<i>Homatula berezowskii</i>	–	NC040302	(Li et al. 2020)
<i>Homatula berezowskii</i>	–	MH125163	(Li et al. 2020)
<i>Homatula change</i>	KIZ:20150252	MF953185	(Endruweit et al. 2018)
<i>Homatula change</i>	KIZ:20150253	MF953186	(Endruweit et al. 2018)
<i>Homatula change</i>	KIZ:20150254	MF953187	(Endruweit et al. 2018)
<i>Homatula change</i>	KIZ:20150255	MF953188	(Endruweit et al. 2018)
<i>Homatula change</i>	KIZ:20150257	MF953189	(Endruweit et al. 2018)
<i>Homatula coccinocola</i>	KIZ:20110001	MF953208	(Endruweit et al. 2018)
<i>Homatula coccinocola</i>	KIZ:2012001866	MF953209	(Endruweit et al. 2018)
<i>Homatula coccinocola</i>	KIZ:2012001867	MF953210	(Endruweit et al. 2018)
<i>Homatula coccinocola</i>	KIZ:2012001868	MF953211	(Endruweit et al. 2018)
<i>Homatula coccinocola</i>	KIZ:2012001869	MF953212	(Endruweit et al. 2018)
<i>Homatula wuliangensis</i>	KIZ:20080581	MF953220	(Endruweit et al. 2018)
<i>Homatula wuliangensis</i>	KIZ:20080582	MF953221	(Endruweit et al. 2018)
<i>Homatula wuliangensis</i>	KIZ:20080584	MF953222	(Endruweit et al. 2018)
<i>Homatula longidorsalis</i>	KIZ:20060274	MF953196	(Endruweit et al. 2018)
<i>Homatula longidorsalis</i>	KIZ:20060276	MF953197	(Endruweit et al. 2018)
<i>Homatula variegata</i>	KIZ:200405015	MF953213	(Endruweit et al. 2018)
<i>Homatula variegata</i>	KIZ:20090009	MF953214	(Endruweit et al. 2018)
<i>Homatula variegata</i>	KIZ:20090010	MF953215	(Endruweit et al. 2018)
<i>Homatula variegata</i>	KIZ:200405045	MF953216	(Endruweit et al. 2018)
<i>Homatula variegata</i>	KIZ:200405046	MF953217	(Endruweit et al. 2018)
<i>Homatula variegata</i>	KIZ:200406167	MF953218	(Endruweit et al. 2018)
<i>Homatula variegata</i>	KIZ:200406168	MF953219	(Endruweit et al. 2018)
<i>Homatula disparizona</i>	KIZ:2012000622	MF953190	(Endruweit et al. 2018)
<i>Homatula disparizona</i>	KIZ:2012000623	MF953191	(Endruweit et al. 2018)
<i>Homatula disparizona</i>	KIZ:2012000624	MF953192	(Endruweit et al. 2018)
<i>Homatula disparizona</i>	KIZ:2012000625	MF953193	(Endruweit et al. 2018)
<i>Homatula disparizona</i>	KIZ:2012000626	MF953194	(Endruweit et al. 2018)
<i>Homatula disparizona</i>	KIZ:2012000627	MF953195	(Endruweit et al. 2018)
<i>Homatula guanheensis</i>	HUN101134	MT771689	(Zhou et al. 2021)
<i>Homatula guanheensis</i>	HUN101135	MT771690	(Zhou et al. 2021)
<i>Homatula guanheensis</i>	HUN101137	MT771691	(Zhou et al. 2021)
<i>Homatula guanheensis</i>	HUN101138	MT771692	(Zhou et al. 2021)
<i>Homatula guanheensis</i>	HUN101139	MT771693	(Zhou et al. 2021)
<i>Homatula guanheensis</i>	HUN101140	MT771694	(Zhou et al. 2021)
<i>Homatula nanpanjiangensis</i>	KIZ 2008006752	MZ677120	(Min et al. 2022)
<i>Homatula nanpanjiangensis</i>	KIZ 2008006749	MZ677119	(Min et al. 2022)
<i>Homatula nanpanjiangensis</i>	KIZ 2009000147	MZ677113	(Min et al. 2022)
<i>Homatula nanpanjiangensis</i>	KIZ 2009000145	MZ677112	(Min et al. 2022)

Taxon	Voucher number	GenBank number	Resource
<i>Homatula cryptoclathrata</i>	KIZ 2005012637	MZ677117	(Min et al. 2022)
<i>Homatula cryptoclathrata</i>	KIZ 2005012639	MZ677116	(Min et al. 2022)
<i>Homatula wenshanensis</i>	KIZ 2014005686	MZ677103	(Min et al. 2022)
<i>Homatula wenshanensis</i>	KIZ 2014005687	MZ677102	(Min et al. 2022)
<i>Homatula laxiclathra</i>	KIZ 2005014383	MG238123	(Min et al. 2023)
<i>Homatula laxiclathra</i>	KIZ 2005014382	MG238122	(Min et al. 2023)
<i>Homutula robusta</i>	KIZ 2009000144	MZ677107	(Min et al. 2023)
<i>Homutula robusta</i>	KIZ 2009000146	MZ677106	(Min et al. 2023)
<i>Homatula gelao</i> sp. nov.	ZVTC20200602	QQ799408	In this study
<i>Homatula gelao</i> sp. nov.	ZVTC20200604	QQ799409	In this study
<i>Homatula gelao</i> sp. nov.	ZVTC20200608	QQ7994010	In this study
<i>Homatula gelao</i> sp. nov.	ZVTC20200609	QQ7994011	In this study
<i>Homatula gelao</i> sp. nov.	ZVTC20200620	QQ7994012	In this study
<i>Homatula gelao</i> sp. nov.	ZVTC20200625	QQ7994013	In this study
<i>Homatula gelao</i> sp. nov.	ZVTC20200626	QQ7994014	In this study
<i>Homatula gelao</i> sp. nov.	ZVTC20200627	QQ7994015	In this study
<i>Homatula gelao</i> sp. nov.	ZVTC20200648	QQ7994016	In this study

KIZ, Kunming Institute of Zoology, Chinese Academy of Sciences. **HUN**, Henan Normal University. - results without voucher numbers. **ZVTC**, Zunyi Vocational and Technical College.



Figure 2. Lateral (A), dorsal (B), and ventral (C) views of *H. gelao*, holotype, ZVTC 20200601, 127.6 mm SL, caught from Furong-Jiang, a tributary flowing into Wu-Jiang of the Yangtze River basin, at Huaiping Village, Daozhen Gelao and Miao Autonomous County, Guizhou Province, P. R. China.

Description. Morphometric measurements were taken from type specimens given in Table 2, and see Fig. 2 for a general appearance of body. Body elongated, anteriorly cylindrical and posteriorly compressed laterally, nearly with uniform depth from behind head to caudal fin base. Head, thorax, and abdomen

scaleless. Dorsum and flanks of predorsal body sparsely covered with tiny scales buried beneath skin. Lateral line complete, with 97–110 perforated scales. Adipose crest extending along dorsal midline of caudal peduncle, anteriorly ends close to above the posterior end of anal-fin base.

Table 2. Morphometric data of *H. gelao* and its related species.

Characters	<i>H. gelao</i>		<i>H. berezowskii</i> (<i>n</i> = 10)	<i>H. variegata</i> (<i>n</i> = 8)	<i>H. oxygnathra</i> (<i>n</i> = 25)
	Holotype	Paratypes Range (Mean ± SD)	Range (Mean ± SD)	Range (Mean ± SD)	Range (Mean ± SD)
SL (mm)	127.56	98.3–135.5 (116.4 ± 10.6)	65.4–125.41 (105.8 ± 30.6)	65.9–122.4 (93.2 ± 16.70)	72.0–129.6 (97.6 ± 16.4)
In percentage of SL					
Body depth	9.8	9.3–13.8 (10.05 ± 0.9)	9.3–12.3 (10.9 ± 1.51)	9.2–12.7 (11.1 ± 1.20)	10.4–14.4 (11.7 ± 1.0)
Head length	16.6	15.0–18.9 (17.1 ± 0.9)	15.6–18.5 (17.2 ± 1.43)	18.7–20.4 (19.60 ± 0.60)	17.6–21.0 (19.8 ± 0.9)
Dorsal-fin length	11.8	11.7–16.7 (13.8 ± 1.3)	9.2–11.2 (9.9 ± 1.01)	6.6–10.3 (8.9 ± 1.1)	11.7–15.1 (13.2 ± 1.0)
Pectoral-fin length	10.9	10.0–13.8 (12.0 ± 0.9)	11.0–13.0 (12.0 ± 1.00)	10.9–13.7 (12.3 ± 0.90)	9.4–12.4 (11.0 ± 0.7)
Ventral-fin length	9.5	9.1–11.7 (10.3 ± 0.7)	10.1–12.6 (10.9 ± 1.29)	10.1–13.1 (11.2 ± 0.90)	11.7–14.9 (13.2 ± 0.7)
Anal-fin length	9.6	10.7–12.6 (11.8 ± 0.6)	7.4–10.8 (8.7 ± 1.72)	7.2–13.7 (10.0 ± 1.90)	12.4–15.1 (13.7 ± 0.7)
Predorsal length	44.0	42.0–46.9 (44.3 ± 1.1)	43.0–47.7 (44.8 ± 2.40)	43.9–48.9 (46.3 ± 1.30)	43.8–48.8 (46.1 ± 1.5)
Prepectoral length	16.6	15.7–19.3 (17. 6 ± 1.0)	15.9–18.9 (17.2 ± 1.51)	16.6–20.6 (18.7 ± 1.30)	16.8–21.7 (20.1 ± 1.2)
Prepelvic length	43.5	43.0–47.7 (45.4 ± 1.1)	42.9–47.5 (45.3 ± 2.30)	44.8–48.6 (46.6 ± 1.00)	44.5–50.0 (47.3 ± 1.5)
Preanal length	73.2	71.1–76.3 (74.1 ± 1.3)	69.2–73.6 (72.1 ± 2.23)	70.90–73.9 (72.5 ± 1.2)	68.2–73.8 (70.9 ± 1.5)
Caudal peduncle depth	9.5	7.9–10.9 (9.2 ± 0.7)	9.1–10.9 (10.0 ± 0.89)	7.8–10.00 (8.6 ± 0.70)	9.0–12.6 (10.3 ± 0.8)
Caudal peduncle length	17.4	16.9–20.8 (19.3 ± 0.9)	18.7–21.7 (20.2 ± 1.52)	20.2–22.4 (21.4 ± 0.70)	17.6–22.8 (20.5 ± 1.3)
In percent of CPL					
Caudal peduncle depth	54.7	40.8–57.6 (48.1 ± 4.2)	42.8–54.4 (49.5 ± 5.85)	36.4–46.6 (40.3 ± 3.40)	45.1–56.0 (50.6 ± 3.0)
In percentage of HL					
Head depth	56. 9	51.5–68.3 (57.1 ± 4.5)	45.3–49.9 (47.8 ± 2.31)	44.5–54.3 (49.54 ± 3.40)	40.6–49.7 (46.5 ± 2.3)
Head width	62.7	56.8–71.0 (62.5 ± 3.6)	56.7–69.0 (61.9 ± 6.17)	52.4–63.5 (58.7 ± 3.10)	52.0–60.3 (55.9 ± 2.2)
Eye diameter	19.5	10.5–20.6 (14.3 ± 2.3)	13.8–16.9 (15.2 ± 1.54)	13.1–15.2 (13.7 ± 0.70)	8.7–13.8 (11.5 ± 1.3)
Snout length	42.9	34.4–51.3 (40.6 ± 4.0)	40.4–46.3 (42.5 ± 3.02)	38.3–42.9 (41.3 ± 1.50)	39.1–45.9 (43.1 ± 1.7)
Interorbital width	33.0	21.7–33.4 (28.5 ± 2.7)	24.1–29.7 (27.0 ± 2.80)	23.0–27.1 (24.8 ± 1.30)	19.3–27.8 (24.8 ± 2.1)

SD, standard deviation. **HL**, head length.

Head small and short, about one-sixth of SL, slightly depressed and wider than deep. Snout pointed in lateral view and pointedly rounded in dorsal and ventral view; eyes small and elliptical, positioned dorsally in the anterior half of head; diameter less than interorbital width. Anterior nostril with a flap-like tube. Mouth inferior and arched; lips slightly thick, fleshy, and non-folded. Three pairs of barbels, two pairs of rostral barbels, and one pair of maxillary barbels; outer rostral barbels longer than inner ones; the inner barbels are invisible when viewed dorsally.

Fins flexible. Dorsal fin with three simple and eight branched rays; distal margin convex; origin closer to snout tip than to caudal fin base. Pectoral fin with two simple and

eight branched rays; tip of pectoral fin extending, not reaching halfway to pelvic-fin insertion. Pelvic fin with 1 and 6¹/₂ branched rays; tip of pelvic fin not extending midway between pelvic-fin origin and anal-fin origin. The insertion point of the ventral fin is directly below the dorsal fin, between the unbranched fin and the first branched fin of the dorsal fin. Anal fin has two simple and five branched rays, with a convex distal margin; origin is closer to caudal fin base than to pelvic-fin insertion. Caudal fin rounded with 21(10+11) branched rays, and the upper lobe slightly longer than lower lobe.

Intestines have no loop, slightly bending at bottom of U-shaped stomach but not Z-shaped, and then extending backwards to posterior part of body (Fig. 5). Air bladder

bipartite; anterior chamber is enclosed in a bony capsule and posterior one degenerated. Vertebrae 4 + 41–43 counted from 5 specimens (Fig. 6).

Coloration. In freshly captured individuals, ground color of head and body is fishy-red, with a dark back and light abdomen. A thick brown-black stripe runs along dorsal midline from behind head to dorsal fin origin, with roughly 7–8 black vertical bars, narrower than interspaces, on either side of this thick brownish-black stripe. 17–18 brown or black vertical bars on flank, including 7–8 on both sides of the predorsal, 3–4 below dorsal-fin base, and 4–5 on caudal peduncle. There are regularly arranged brownish-black spots on the base and tip of the dorsal fin and a dark brown vertical bar on caudal-fin base. Caudal fin dark fishy red, sparsely covered with small black spots. All other fins yellowish transparent (Figs 2, 3A, 4A).

In formalin-preserved specimens, back and flank are earthy yellow, and abdomen is slightly yellowish. All fins milky white, except for black spots on dorsal and caudal fins. Vertical bars on flank paler than those of freshly collected specimens (Fig. 7A, B).

Distribution and habitat. *Homatula gelao* is currently known only from the Huaixi-He, a stream tributary to the Furong-Jiang. The type specimens were captured in a 6 km main channel length of the Huaixi-He, with an altitude of 600 to 700 m above sea level. *H. gelao* was commonly found at the bottom of underwater gravels with swirling currents and the larvae of benthic invertebrates such as Ephemeroptera, Trichoptera, and Plecoptera (Figs 8, 9). Coexisting species are *A. furongjiangensis* (Xiao, 2024), *S. szechuanensis* (Fang, 1930), *M. anguillicaudatus* (Cantor, 1842), *P. prochilus* (Sauvage et Dabry, 1874), and *S. kozlovi* (Nikolsky, 1903).

Sexual dimorphism. No sexual dimorphism was observed.

Etymology. The specific epithet is named after *Gelao* (in Chinese spelling) nationality. The type specimens of the new species were collected from the Huaixi-He in Daozhen Gelao Autonomous County, northern Guizhou Province. The Chinese common name for *H. gelao* is suggested as “佬佬荷马条鳅”.



Figure 3. The extension characteristics of adipose crest forward to the vertical via the posterior end of the anal-fin base and the shape of caudal fin shapes of *H. gelao* (A), *H. variegata* (B), *H. oxygnathra* (C), and *H. berezowskii* (D) (B–D, copy from Liu et al. 2022).

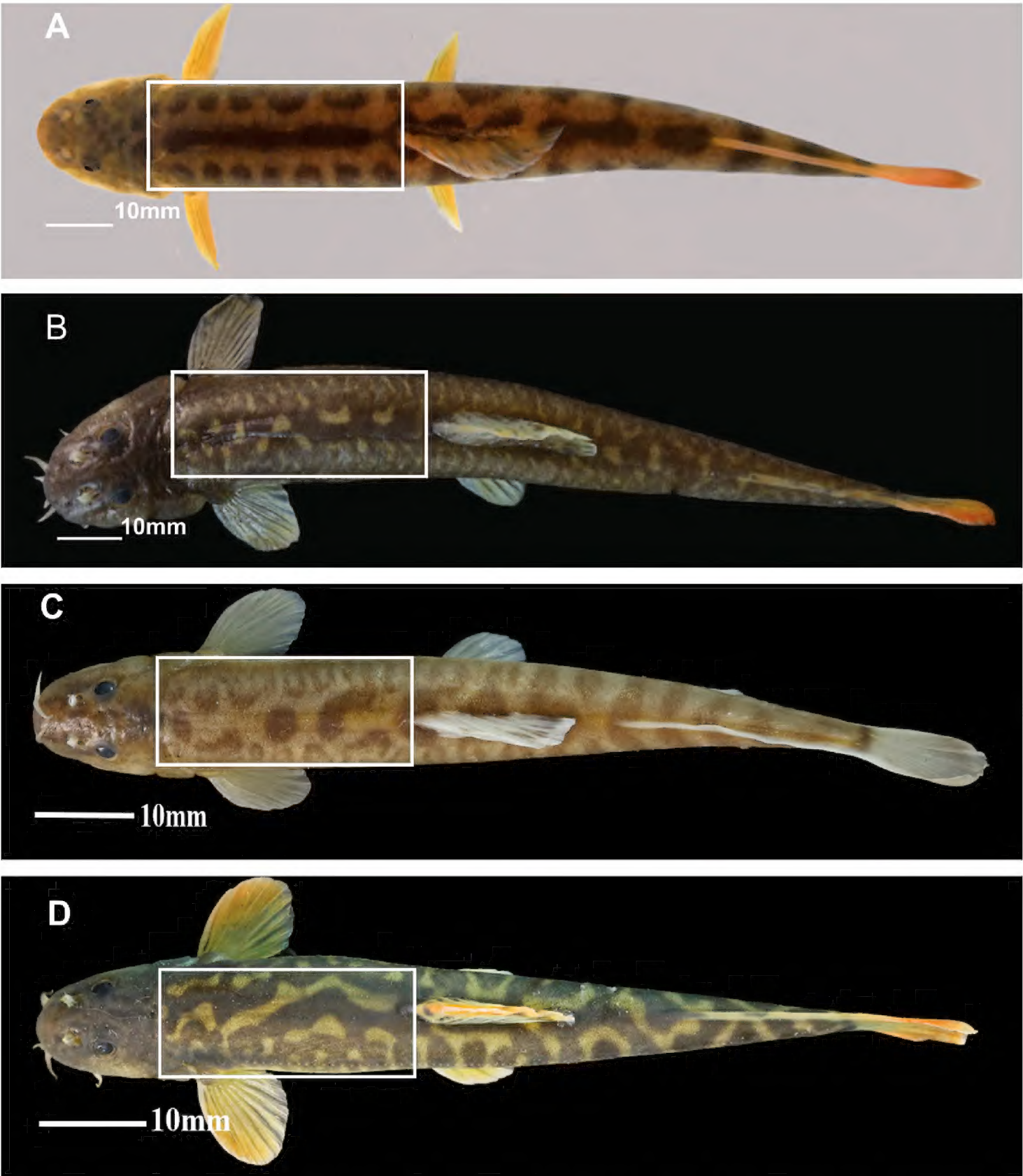


Figure 4. Stripes of the anterior part of the predorsal fin of body of *Homatula gelao* (A), *Homatula variegata* (B), *H. oxygnathra* (C), and *H. berezowskii* (D) (B–D, copied from Liu et al. 2022, and slightly modified).

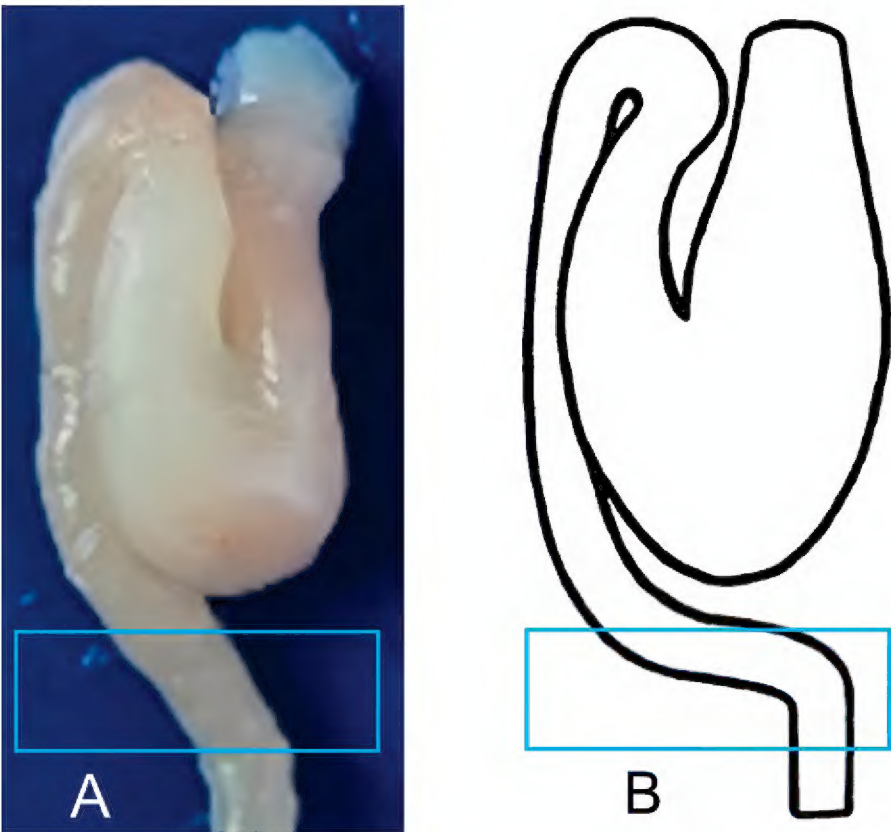


Figure 5. Shape pattern of intestinal (blue square) for *H. gelao* (A) and *Homatula variegata* (quoted from Liu et al. 2022) (B).

Molecular phylogeny and genetic distance

The mitochondrial COI gene sequences, with a length of 649 bp, were identified for 11 samples of *H. gelao*. After aligning with species of the same *Homatula* genus, there are 560 conserved sites, 15 variable sites, 3 parsimonious informative sites, and 12 singleton sites. The nucleotide composition of this gene is A = 21.7%, T = 29.2%, C = 27.9%, and G = 21.1%. The average G + C content varied from 48.9% to 49.5%, with an average value of 49.2%. The resulting phylogenetic tree, yielded based on the mtDNA COI genes using the neighbor-joining method, is depicted in Fig. 10. The topology of this tree reveals that samples of *H. gelao* clustered together into a highly supported distinct lineage. The phylogenetic analysis indicates that *H. gelao* constitutes a sister group to the clade comprising ((*H. guanhensis* + *H. laxiclathra*) + ((*H. longidorsalis* + *H. robusta*) + (*H. nanshanjiangensis* + *H. variegata*))). The genetic distances between the sampled species of *Homatula* ranged from

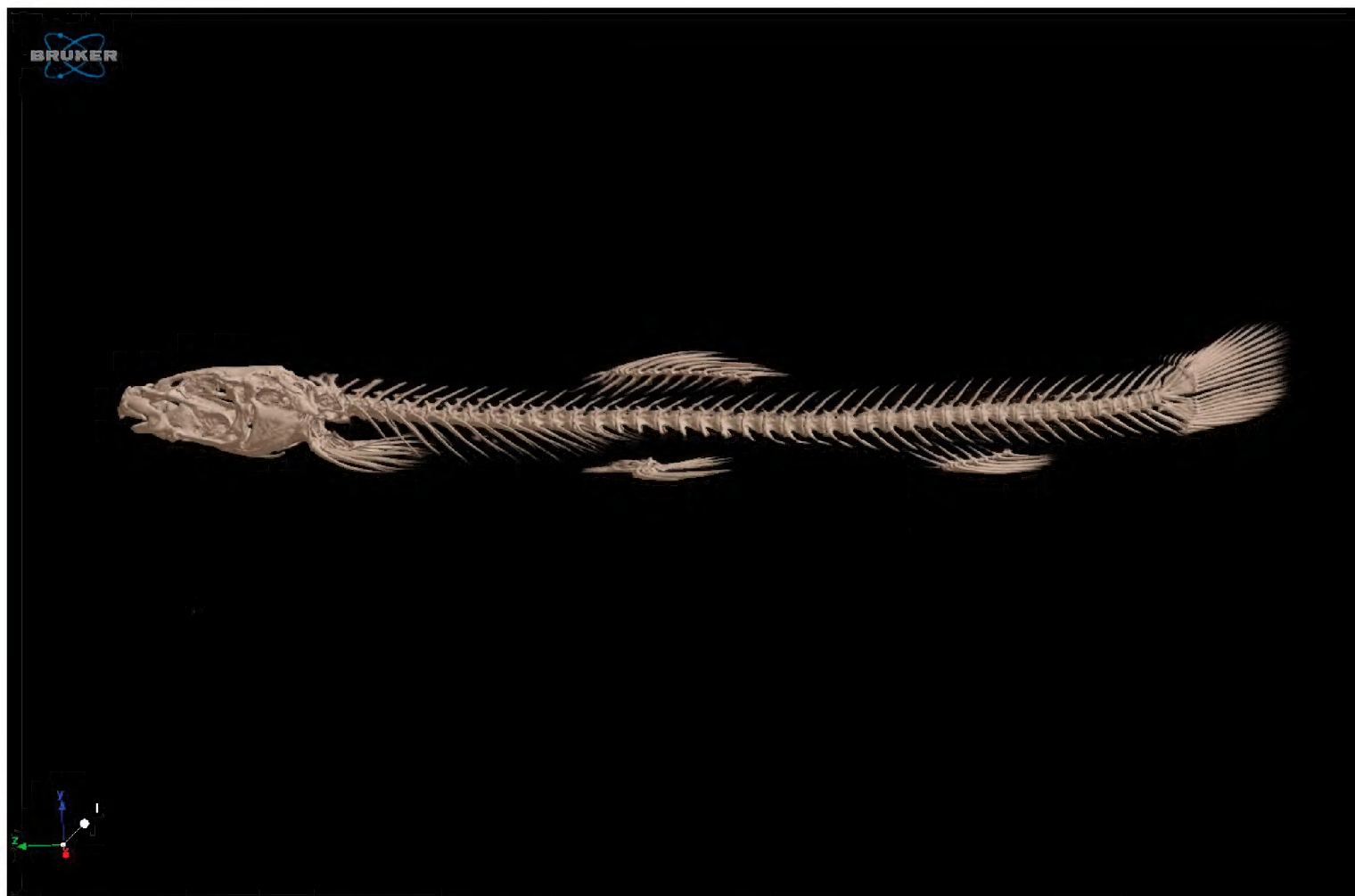


Figure 6. Micro-CT scanned image of body in lateral view for *H. gelao*.



Figure 7. The specimens of steeping in formalin of *H. gelao* (A) dorsal view; (B) ventral view.



Figure 8. Sampling locality of *H. gelao* in the Huaixi-He, tributary to the Furong-Jiang in Huaiping Village, Jiucheng Town, Daozhen Gelao and Miao Autonomous County, Zunyi City, Guizhou Province, P. R. China; 2 June 2020, photographed by Guibang Xiao.

8.21% to 12.19%. The average genetic distance is $9.92\% \pm 1.08\%$, and the interspecific genetic distance is much greater than 2% (Table 3).

Comparative morphometry

The results demonstrated that $KMO = 0.738 > 0.70$, and Bartlett’s $P < 0.01$, indicating that the morphological traits used in this study are appropriate for PCA analysis (Kaiser 1974; Schreiber 2021). PCA was performed for 17 morphometric measurements from 28 specimens of *H. gelao* and 8 specimens of *H. variegata*. The results showed that in the scatterplot of PC1 against PC2 and PC2 against PC3, two distinct clusters representing *H. gelao* and *H. variegata* were identified (Fig. 11). Specimens of *H. gelao* were separated from specimens of *H. variegata* along the PC1 and



Figure 9. The view of perched states of a fresh individual of *H. gelao* in Daozhen Gelao and Miao Autonomous County, Guizhou, P. R. China. The white arrow indicates the backwater areas. Red arrow indicates the natural habitat state of *H. gelao*. White square indicates the inhabitant situation of *H. gelao*.

Table 3. Genetic distances between *H. gelao* and its congeneric species.

		1	2	3	4	5	6	7	8	9
<i>H. gelao</i>	1		0.0136	0.0153	0.0139	0.0129	0.0143	0.0134	0.0129	0.0127
<i>H. anguillioides</i>	2	0.103		0.0118	0.011	0.0118	0.0079	0.0131	0.0126	0.0121
<i>H. berezowskii</i>	3	0.1186	0.0802		0.0122	0.0131	0.0119	0.0129	0.013	0.0135
<i>H. change</i>	4	0.1062	0.0716	0.0907		0.0096	0.0122	0.0129	0.0121	0.0117
<i>H. coccinocola</i>	5	0.1016	0.0772	0.1068	0.0554		0.0124	0.0132	0.0124	0.0122
<i>H. cryptoclathrata</i>	6	0.108	0.0391	0.0762	0.0779	0.0866		0.013	0.0132	0.0128
<i>H. disparizona</i>	7	0.1016	0.0954	0.0922	0.0928	0.0985	0.0975		0.0132	0.0127
<i>H. guanhensis</i>	8	0.0875	0.0919	0.0942	0.0825	0.0884	0.0981	0.086		0.0051
<i>H. laxiclathra</i>	9	0.0885	0.0883	0.104	0.0797	0.0832	0.0948	0.0874	0.0168	
<i>H. longidorsalis</i>	10	0.0821	0.0733	0.0855	0.0666	0.0747	0.0854	0.0808	0.0509	0.048
<i>H. nanpanjiangensis</i>	11	0.0839	0.0789	0.0878	0.0752	0.0747	0.08	0.0767	0.0619	0.0593
<i>H. potanini</i>	12	0.1236	0.0808	0.0088	0.0943	0.1074	0.0783	0.0928	0.0928	0.1025
<i>H. pycnolepis</i>	13	0.0996	0.0364	0.0802	0.0752	0.0807	0.0425	0.0981	0.0887	0.0896
<i>H. variegata</i>	14	0.0975	0.0792	0.0903	0.0694	0.0789	0.0922	0.086	0.0534	0.0468
<i>H. wenshanensis</i>	15	0.1077	0.0854	0.1006	0.1009	0.092	0.0903	0.0586	0.0879	0.0933
<i>H. wuliangensis</i>	16	0.1072	0.0869	0.1127	0.0743	0.0879	0.0872	0.104	0.0819	0.0788
<i>H. robusta</i>	17	0.0925	0.076	0.0953	0.0835	0.083	0.0833	0.087	0.0655	0.0586
		10	11	12	13	14	15	16	17	
<i>H. gelao</i>	1	0.0114	0.0121	0.0154	0.0132	0.0134	0.0135	0.0132	0.0127	
<i>H. anguillioides</i>	2	0.0108	0.0116	0.0116	0.0081	0.0111	0.0124	0.0123	0.0116	
<i>H. berezowskii</i>	3	0.0119	0.0119	0.0028	0.0119	0.0123	0.0139	0.0145	0.0126	
<i>H. change</i>	4	0.0107	0.0112	0.0123	0.0115	0.0107	0.0135	0.0112	0.0122	
<i>H. coccinocola</i>	5	0.0113	0.011	0.0129	0.0116	0.0116	0.0127	0.0124	0.0121	
<i>H. cryptoclathrata</i>	6	0.0118	0.0116	0.0119	0.0085	0.012	0.0123	0.0124	0.0121	
<i>H. disparizona</i>	7	0.012	0.0117	0.0128	0.0138	0.0126	0.0103	0.0144	0.0129	
<i>H. guanhensis</i>	8	0.0088	0.01	0.0127	0.0126	0.0089	0.013	0.0116	0.0104	
<i>H. laxiclathra</i>	9	0.0086	0.0097	0.0131	0.0123	0.0084	0.0131	0.0115	0.01	
<i>H. longidorsalis</i>	10		0.0077	0.0117	0.0114	0.0069	0.0124	0.0112	0.0078	
<i>H. nanpanjiangensis</i>	11	0.0444		0.0119	0.012	0.008	0.0122	0.0121	0.0086	
<i>H. potanini</i>	12	0.0859	0.0884		0.0117	0.0121	0.0138	0.0146	0.0126	
<i>H. pycnolepis</i>	13	0.0749	0.084	0.0808		0.012	0.0124	0.0121	0.012	
<i>H. variegata</i>	14	0.0392	0.0464	0.0909	0.083		0.0127	0.0104	0.0086	
<i>H. wenshanensis</i>	15	0.0853	0.0826	0.1013	0.0882	0.0907		0.0126	0.0131	
<i>H. wuliangensis</i>	16	0.0737	0.0864	0.1144	0.0841	0.0707	0.094		0.0123	
<i>H. robusta</i>	17	0.0422	0.0533	0.0959	0.0812	0.0513	0.0902	0.0877		

The numbers 1 to 17 correspond to the congeneric species of *Homatula* in the left column of the table. The numbers of the upper right triangle represent the standard deviation.

PC3 axes, respectively. There are two morphometric measurements (head length and prepectoral length) with main loadings on the first axis (PC1), three (dorsal-fin length, anal-fin length, and caudal peduncle depth) on PC2, and two (head depth and body depth) on PC3. Characters with main loading included head length, prepectoral length, head depth, and body depth. Major differences were found in head length (15.0–18.9, mean 17.1 in *H. gelao* vs. 18.7–20.4, mean 19.60 in *H. variegata*, % of SL), caudal peduncle length (16.9–20.8, mean 19.3 in *H. gelao* vs. 20.2–22.4, mean 21.4 in *H. variegata*, % of SL), and dorsal fin length (11.7–16.7, mean 13.8 in *H. gelao* vs. 6.6–10.3, mean 8.9 in *H. variegata*, % of SL) (Table 4).

Table 4. Loading on the first three principal components for each morphometric measurement for *H. gelao* (n = 28, 98.3–135.5 mm SL) and *H. variegata* (n = 8, 65.9–112.4 mm SL).

	PC1	PC2	PC3
Body depth	0.147	0.176	0.541
Head length	0.373	0.020	-0.052
Dorsal-fin length	-0.243	0.334	-0.104
Pectoral-fin length	0.193	0.323	-0.150
Pelvic fin length	0.283	0.215	-0.208
Anal-fin length	-0.158	0.337	0.082
Predorsal length	-0.323	0.249	-0.069
Prepectoral length	0.352	-0.182	0.0513
Prepelvic length	0.248	0.203	-0.009
Preanal length	-0.144	0.270	-0.140
Caudal peduncle depth	-0.030	0.326	0.150
Caudal peduncle length	0.260	-0.238	0.0146
Head depth	0.073	0.256	0.543
Head width	0.282	0.192	0.245
Eye diameter	0.242	0.216	-0.266
Snout length	0.308	0.065	-0.109
Interorbital width	0.144	0.259	-0.367
Cumulative variance (%)	76.017	87.302	90.865

Discussion

Homatula gelao is characterized by the possession of a complete lateral line, a scarcely scaled or scaleless pre-dorsal body, and a slender body with a uniform depth. The three characters place the new species in the elongate-bodied group of *Homatula* defined by Liu (Liu et al. 2022), which includes *H. berezowskii*, *H. guanheensis*, *H. laxiclathra*, *H. longidorsalis*, *H. oxygnathra*, *H. tigris*, and *H. variegata*. *H. gelao* was previously misidentified as *H. variegata* (Yang 2022). The latter has recently been shown to have a restricted distribution in the Wei-He of the middle Huang-He basin (Liu et al. 2022). There are significant differences in body coloration, head shape, and caudal-peduncle thickness (see the diagnosis for details).

H. gelao is currently found in the Wu-Jiang of the upper Yangtze River basin. Two other congeneric species coexisting in the Wu-Jiang are *H. potanini* and *H. wujiangensis* (Liu 2022). The new species is distinct from both in having, among others, a complete (vs. incomplete) lateral line, an adipose crest along the dorsal midline of the caudal

peduncle that anteriorly ends close to above the posterior end (vs. prior to the anterior end in *H. potanini* or posterior to the posterior end in *H. wujiangensis*) of the anal-fin base, scales sparsely scattered over the pre-dorsal body (vs. the caudal peduncle in *H. wujiangensis*), a broadly rounded (vs. truncate) caudal fin, and a slender (vs. stout) caudal peduncle (length 16.9–20.8% of SL vs. 12.8–13.8 in *H. potanini* and 13.5–15.1 in *H. wujiangensis*).

Recently, *H. tigris* was described as a new species from the Panlong-He, a stream tributary to the Jinsha-Jiang in Yunnan Province (Che et al. 2023). This new species is distinct from *H. gelao* in having, among others, a sparsely scaled (vs. fully scaled) pre-dorsal body, an adipose crest along the dorsal midline of the caudal peduncle anteriorly extending to the vertical close to the posterior end of the anal-fin base (vs. the anterior end) of the anal-fin base, and a body coloration of 17–18 black vertical bars, narrower than interspaces (vs. 18–24 dark brown vertical bars, wider than interspaces) on each side of the body.

In fish, mechanosensation is mostly sensed through the lateral line on the skin (Yoffe et al. 2020). It plays an important role in aspects such as detection of prey (Liang et al. 1998; Coombs 1999; Pohlmann et al. 2004), evasion of predators (Blaxter and Fuiman 1990), avoidance of obstacles (Abdel-Latif et al. 1990; Hassan 1992), mating (Satou et al. 1994), schooling (Partridge and Pitcher 1980; Partridge 1981), and rheotaxis (Montgomery 1997; Baker and Montgomery 1999; Suli et al. 2012). The *H. gelao* having a complete lateral line (vs. incomplete in *H. potanini* and *H. wujiangensis*). This observation implies that both prey and predators within the habitat of *H. gelao* exhibit agile movements, suggesting that the habitat of *H. gelao* is frequently turbulent. Consequently, the habitat of *H. gelao* is distinct from that of *H. potanini* and *H. wujiangensis*; however, further studies are needed to elucidate the specific differences between them.

Previous studies have not mentioned the presence characteristics of the striation of the predorsal body of the genus *Homatula*. We found that there are differences in these characteristics between different congeners of *Homatula*. In particular, *H. gelao* has a dark brown longitudinal stripe in the middle of the back above the dorsal fin, which can be used to distinguish it from other congeneric species (Fig. 4). Studies have shown that stripe patterns, prominent in many animals, have important functions such as camouflage, kin recognition, shoaling, and sexual selection (Rosenthal and Ryan 2005; Frohnhofer et al. 2013), and it can be inferred that the *H. gelao* may be different in the above aspects from other congeneric species.

Molecular phylogenetic analysis has shown that the *H. gelao* is the sister group to a clade consisting of *H. guanheensis*, *H. laxiclathra*, *Homatula longidorsalis*, *Homatula robusta*, *Homatula nanpanjiangensis*, and *Homatula variegata*. However, *H. gelao* was in the dominant position, suggesting that *H. gelao* evolution was earlier than other species in the clade of the sister group. Furthermore, *H. gelao* was in different clades from *Homatula wujiangensis* and *Homatula potanini*.

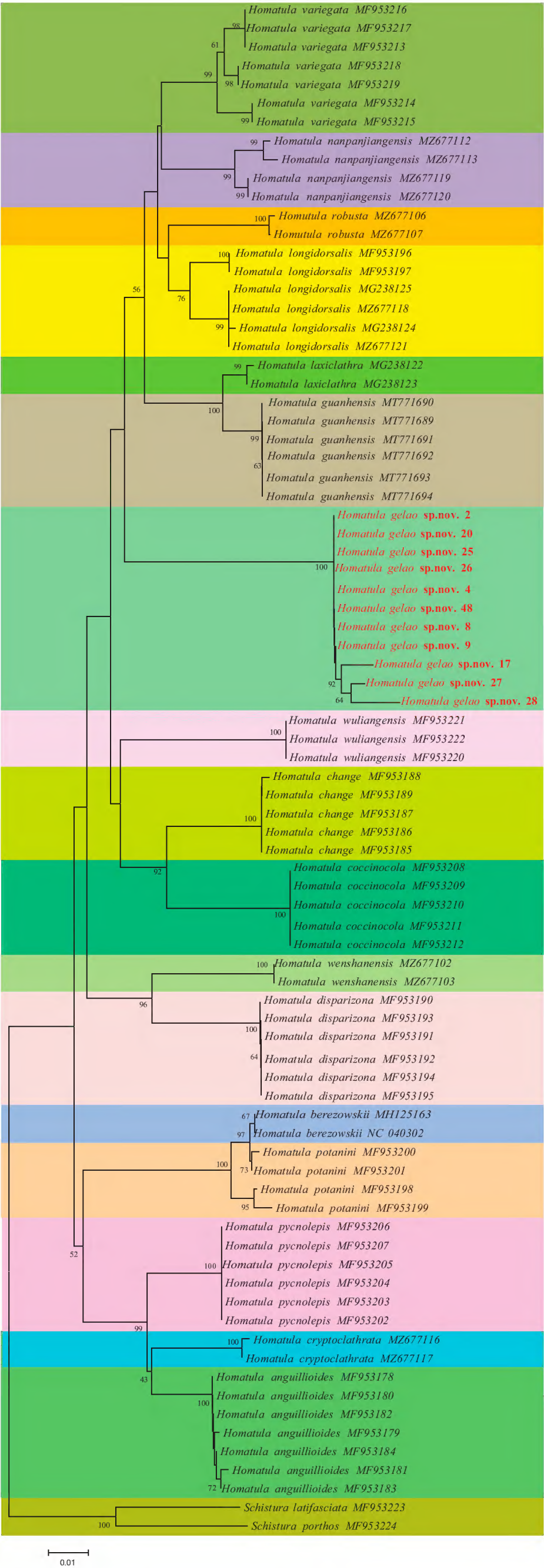


Figure 10. The phylogenetic tree of the loaches of *Homatula* based on the COI gene; *Schistura latifasciata* and *Schistura porthos* were used as the outgroups.

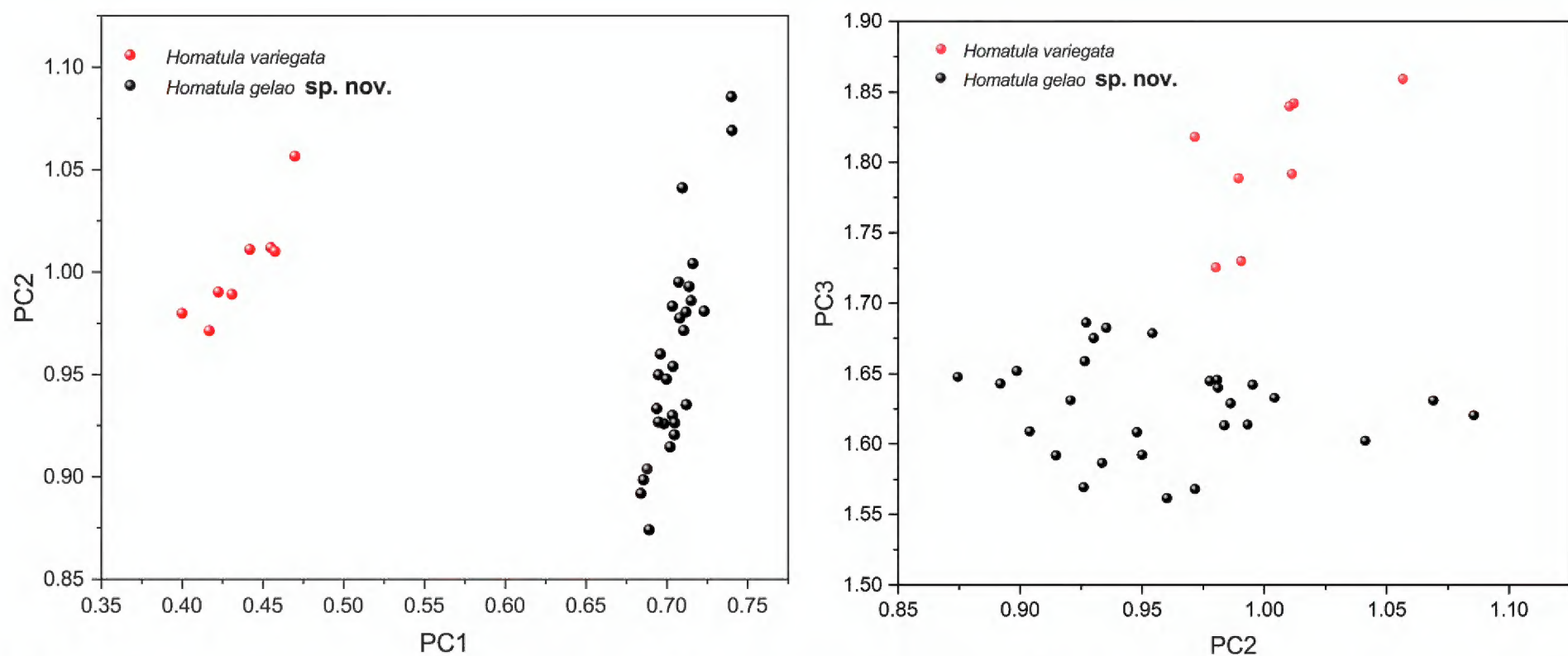


Figure 11. Scatter plots of the first and second (left) and second and third (right) principal components from morphometric data of type specimens of *H. gelao* (black balls) and *H. variegata* (red balls).

A 2% genetic distance threshold is typically employed to distinguish between the majority of species (Hebert et al. 2003). Furthermore, Hebert et al. (2004) proposed that species can be distinguished when the average interspecific genetic distance was 10 times greater than the average genetic distance between intraspecific of its congeneric species. Based on a K2P genetic distance analysis of the mitochondrial COI genes, the genetic divergence between *H. gelao* and *H. variegata*, as well as that between *H. gelao* and *H. berezowskii*, reaches 9.75% and 11.86%, respectively, which are greater than 2%. Meanwhile, the interspecific average genetic distance between *H. gelao* and its congeneric species is 18 times the genetic intraspecific distance of *H. gelao* (Suppl. material 1), the validity of the new species was further supported.

Comparative materials

***Homatula variegata*.** IHB 202106049914–9917, 202106055611–5614, topotype, 8 specimens, 65.9–122.4 mm SL, China: Shaanxi Province, Xi'an City, Dayu-He, a tributary emptying into Wei-He of Huang-He basin.

***Homatula berezowskii*.** IHB 82V2385–2386, topotype, 2 specimens, 116.5–86.9 mm SL; China: Gansu Province, Hui County. IHB 82VI2418, 82V2290–2293, 73VI1044–1045, 7 specimens, 58.2–122.5 mm SL; China: Gansu Province, Feng County. IHB 64VI0135–0139, 64VI0601–0603, 78V0484–0485, 10 specimens, 74.6–128.0 mm SL; China: Gansu Province, Wen County. IHB 92VI2749–2757, 9 specimens, 97.9–129.7 mm SL; China: Gansu Province, Wudu County. IHB 82VI2489, 1 specimen, 126.1 mm SL; China: Gansu Province, Cheng County. IHB 82VI2547–2550, 4 specimens, 72.4–88.4 mm SL; China: Gansu Province, Kang County. IHB 80VI0869–0870, 80VI0874, 80VI0876–0881, 9 specimens, 96.9–155.9 mm SL; China: Shaanxi Province: Fuoping County. IHB 80VI1032, 1 specimen,

89.7 mm SL; China: Shaanxi Province: Ningshan County. IHB 80VII1403, 1 specimen, 100.5 mm SL; China: Shaanxi Province: Shanyang County. IHB 73VI1191, 73VI1194, 2 specimens, 90.9–125.7 mm SL; China: Shaanxi Province: Jieyang County. IHB 73VI1075, 1 specimen, 67.3 mm SL; China: Shaanxi Province: Baishu-Jiang of Jialing-Jiang. IHB 80VI1237–1241, 80VI1185, 80VI1173–1174, 80VI1176–1177, 80VI1181–1182, 12 specimens, 50.9–120.6 mm SL; China: Shaanxi Province: Zhenba County.

***Homatula oxygnathra*.** BMNH-1908.2.27.23, lectotype, 1 specimen, 113.3 mm SL; China: Yunnan Fu (= Kunming City) (photograph examination). BMNH-1908.2.27.24, paralectotype, 1 specimen, 86.7 mm SL; China: Yunnan Fu (= Kunming City) (photograph examination). IHB 201909035805–5819, 15 specimens, 72.0–129.6 mm SL; China: Sichuan Province, Yanbian County, upper Chang-Jiang basin. IHB 201909035461–5470, 10 specimens, 86.7–122.4 mm SL; China: Yunnan Province, Yuanmou County, upper Chang-Jiang basin.

***Homatula wujiangensis*.** IHB 2017053707–3710, lectotype, 4 specimens, 40.5–76.3 mm SL, Chongqing Province: Nanchuan County: Jingfo mountain, Wu-Jiang flowing into upper Chang-Jiang basin.

***Homatula potanini*.** IHB 42IX0661–2, 42IX0664, 42IX0666–7, 79IV0597–8, 79IV0600, 79IV0605, 79IV0609–10, 82V0301–4, 15 specimens, 68.6–83.3 mm SL, China: Sichuan Province: Emei County: Qingyi-Jiang of upper Chang-Jiang basin.

***Homatula guanheensis*.** IHB 202106049918–9921, 202106055625–5627, 7 specimens, 65.7–95.0 mm SL; China: Shaanxi Province, Fuoping County. IHB 202106049922–9923, 202106055640–5642, 5 specimens, 72.4–135.9 mm SL; China: Shaanxi Province, Langao County.

***Homatula laxiclathra*.** IHB 73 V10738, holotype, 136.7 mm SL; China: Shaanxi Province, Zhouzhi

County, Wei-He basin. IHB 80VI0957, 82VI0103, 82VI0106–0108, 80VI0965–0968, 80VI0972, paratypes, 10 specimens, 75.5–136.6 mm SL; China: Shaanxi Province, Zhouzhi County, Wei-He basin. IHB 202106049909–9912, 202106055611–5619, topotype, 8 specimens, 74.7–127.5 mm SL; China: Shaanxi Province, Zhouzhi County, Dong-He into Wei-He. IHB 202106049899–9908, 10 specimens, 75.5–136.6 mm SL; China: Shaanxi Province, Taibai County, Shitou-He into Wei-He; IHB 202106049888–9893, 202106049898, 7 specimens, 65.6–131.3 mm SL; China: Shaanxi Province, Mei County, Bawang-He into Wei-He.

***Homatula longidorsalis*.** IHB 202006047694, 1 specimen, 84.0 mm SL, China: Yunnan Province, Zhanyi County, Niulan-Jiang of upper Chang-Jiang basin.

***Homatula pycnolepis*.** IHB 814042–3, 814045, 814046, 814047 (stained), 814048–51, 9 specimens, 90.5–1118.8 mm SL; China: Yunnan Province: Jianchuan County: Yangbi River, a tributary of upper Mekong, at Shaxi Town; about 26°19'N, 99°51'E; 2100 m above sea level; J. x. Chen, Sept 1981.

Ethics statement

This study was approved by the Ethics Committee of Sichuan University. All animal experiments comply with the ARRIVE guidelines and are carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Data availability statement

The COI gene sequence of *H. gelao* has been submitted to GenBank under the submission ID of SUB13057802. The sequence IDs are [OQ799408–OQ799416](#). We have requested that the sequence data be released on May 31, 2026.

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Author Contributions statement

Ding-Zhi Fang and Gui-Bang Xiao conceived the ideas and designed the methodology; Gui-Bang Xiao, Liang Cao, Qi-Qi Xiao, Lin Zhou, and Hao-Ran Zhang collected the data; Gui-Bang Xiao, Qi-Wei Guo, Liang Cao, Jia Lin, and Wei-Xi Deng analyzed the data; Qi-Wei Guo, Jia Lin, and Ding-Zhi Fang led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Supplementary material 1

Pairwise comparisons of intra-specific genetic distances of species based on Kimura's two-parameter model generated from the sequences of COI gene

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Data type: docx

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